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Immunophenotypic and molecular comparison between allogeneic and autologous graft-versus-host disease of the skin: A retrospective study using proteomics methods

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Graft-versus-host disease (GVHD), a potentially devastating complication of hematopoietic cell transplantation (HCT), occurs when the transplanted immune system recognizes host tissues as being foreign and launches a cytotoxic immunologic attack, resulting in damage to affected tissues.^{1,2} Interferon-mediated processes are implicated in the early pathogenesis of GVHD.^{3,4} Though transplantation of autologous hematopoietic cells mitigates risk for GVHD, a syndrome resembling acute allogeneic GVHD may occur [autologous GVHD (auto-GVHD); auto-aggression syndrome; early engraftment syndrome].^{5–7} Though prior studies have investigated the role of T-cell immunity in auto-GVHD^{7,8}, this syndrome remains poorly understood and somewhat controversial.⁹ It is conceivable that minor immunologic alterations in the host during conditioning or the graft during graft preparation may permit a GVHD-like phenomenon to unfold, though this hypothesis is unsubstantiated. We are unaware of previous work directly comparing the molecular profiles of allo-GVHD and auto-GVHD, and it remains unclear whether these represent identical biologic processes. We aimed to clarify the immunophenotypic and molecular relationship, if any, between acute allo-GVHD and auto-GVHD using immunohistochemical and proteomics methods.

After obtaining institutional review board approval and an informed consent waiver due to the minimal risk nature of this retrospective study, we selected all skin biopsies with changes of auto-GVHD (1994–2012) and compatible clinical features, as per meticulous medical record review. We selected twice as many cases of acute allo-GVHD, matched by histopathologic grade. For controls, we used normal skin (re-excision specimen tips or biopsies with negligible histopathologic changes) from patients who had undergone HCT and never developed GVHD. Tissue samples were excluded if blocks were exhausted or if too few proteins could be identified.

We used a laser capture microdissection-assisted liquid chromatography-based tandem mass spectrometry method modified from those described previously.¹⁰ Specifically, we mounted 10µm thick sections of formalin-fixed, paraffin-embedded skin specimens onto polyethylene naphthalate membrane slides and stained them with Congo Red stain (Sigma-Aldrich, St. Louis, MO) for microanatomic visualization. We performed laser capture microdissection to isolate the mid-granular layer of the epidermis to the superficial dermis, and we dissected 1,000,000µm² of tissue per case, using adhesive cap tubes for collection (Zeiss). For quality purposes, we repeated analysis of all of the auto-GVHD cases, one of the allo-GVHD cases, and one of the control cases. We recorded proteins that were differentially expressed (>20-fold expression; $p < 0.05$; false discovery rate < 1%) between each comparison group. We also performed a limited panel of immunohistochemical stains to further explore the T-cell subsets (CD3, CD4, CD8, FoxP3).

We included 9 cases (allo-GVHD, $n=6$; auto-GVHD, $n=3$) and 3 controls (Table 1). The average number (range) of distinct peptides expressed was 8,863 (5,822–11,187) in the auto-GVHD group, 8,304 (4,024–11,873) in the allo-GVHD group, and 5,573 (4,350–6,536) in the control group. Table 2 documents the 12 proteins (0.3% of all detected) that were overexpressed in auto-GVHD compared with normal skin. Of these proteins, all (100%) were also overexpressed in allo-GVHD, as were 14 additional proteins (data not shown). No protein was underexpressed in auto- or allo-GVHD relative to controls. Within the junctional and intraepidermal CD3-positive lymphocyte population, immunohistochemistry revealed a slight CD8-predominance over CD4, with a paucity of FoxP3 expression in all cases of auto-GVHD and allo-GVHD (Figure).

Each of the 12 mutually overexpressed proteins fell into one of 4 biological categories: (1) interferon-induced, (2) antigen processing and presentation, (3) cellular adhesion or (4) response to cellular stress. The prominence of interferon-induced proteins and antigen processing and presentation identified in this study support the current understanding of interferon's key role in, and the importance of antigen presentation for, pathogenesis. Moreover, the interferon protein signature corresponds with the vacuolar interface changes seen microscopically in skin biopsies of GVHD.¹¹ We hypothesize that adhesion molecules were liberated during the keratinocyte apoptosis that occurs in the setting of GVHD and that proteomic respondents to cellular stress were overexpressed in association with tissue injury. The additional 14 proteins overexpressed in allo-GVHD did not appear to show consistent biologic functions but could be the subject of future study.

This study is preliminary and is limited by the small sample size, attributable to the rarity of auto-GVHD and the labor-intensive methods associated with proteomic analysis. Though control tissue was derived from patients who had previously undergone HCT in an attempt to control for transplant-related variables, it is possible that other variations in conditioning regimen, GVHD prophylaxis regimen, or other patient-related factors may have confounded findings. Finally, without there being a gold-standard for the diagnosis of GVHD, we relied on careful clinicopathologic correlation by a multidisciplinary team at the time of patient care and retrospective chart review for case selection.

Comparison of the proteomic profile of auto-GVHD with that of allo-GVHD, as well as the T-cell populations by immunohistochemistry, revealed striking similarities, thereby implying some overlap in pathogenesis. It is also possible that these proteins may be overexpressed non-specifically in vacuolar interface dermatoses, which could be explored in future studies.

This study used a unique, non-biased approach to investigate the protein milieu of skin affected by GVHD. The results not only validated the importance of interferon induction and antigen presentation in GVHD but also identified several additional proteins of interest that appear not to have been studied in GVHD previously. Future investigations could assess the utility of these proteins as potential tissue-based biomarkers for diagnosis or prognosis of GVHD.

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Figure.

Autologous GVHD (a–e) and allogeneic GVHD (f–j), showing a slight predominance of CD8 over CD4 amongst junctional and epidermal CD3-positive T-lymphocytes and sparse FoxP3-positive cells. a, f: hematoxylin and eosin, 20 × original magnification; b, g: CD3, 20 × original magnification; d, i: FoxP3, 20 × original magnification; e, j: CD8, 20 × original magnification.

Table 1

Clinical and skin histopathologic presentation of patients with auto-GVHD, allo-GVHD or negative control

	Allogeneic GVHD				Autologous GVHD				Controls		
	F	M	M	M	F	M	F	M	M	M	M
Sex											
Age at time of biopsy	36.5	31.0	41.9	37.7			27.4		70.1	60.5	62.1
Days after HCT at time of biopsy	111	33		116			83		38	142	41
Hematologic diagnosis	AML	PNH	ALL, then therapy-related AML	CML			AML		MM	MM	MM
Type of HCT	Allo, MUD	Allo, MRD	Allo, MRD	Allo, MRD			Allo, double umbilical cord blood		Auto	Auto	Auto
Conditioning regimen	Fludarabine and busulfan	Cyclophosphamide and ATG	Cyclophosphamide and TBI	Cyclophosphamide and TBI			Fludarabine, cyclophosphamide, TBI		Melphalan	Melphalan	Melphalan
GVHD prophylaxis	MTX and tacrolimus	Cyclosporine	Cyclosporine	MTX and cyclosporine			Mycophenolate mofetil and cyclosporine		None	None	None
Organs involved by GVHD	Skin	Skin, liver	Skin	Skin			Skin, GI		Skin, GI	Skin, GI	Skin, GI
Duration of follow-up after HCT (days)	1472	176	3590	5586			484		99	22672	43
Clinical outcome	Deceased (AML relapse)	Deceased (cause not documented)	Alive	Alive			Deceased (AML relapse)		Deceased (failure to thrive)	Deceased (cause not documented)	Deceased (complications of MM)
Results of CMV testing (blood PCR) at time of rash	Negative	Negative	Negative	Not done			Negative		Negative	Negative	Negative
Skin histopathology grade	2	2	1	2			2		2	1	2
									N/A	N/A	N/A

F=female; M=male; MM=multiple myeloma; CML=chronic myelogenous leukemia; ALL=acute lymphoblastic leukemia; AML=acute myeloid leukemia; PNH=paroxysmal nocturnal hemoglobinuria; allo=allogeneic; MUD=matched unrelated donor; MRD=matched related donor; GI=gastrointestinal; NA=not applicable

Table 2

Name of proteins significantly overexpressed ($>20 \log^2$ fold) in skin sample, by specimen type, compared to negative control

	Auto-GVHD	Allo-GVHD	
Protein name	Log ² fold-overexpression vs. negative control	Log ² fold-overexpression vs. negative control	Protein function/relevance to GVHD, if known
Interferon-induced			
interferon-induced GTP-binding protein Mx1	30.1	29.8	Strongly induced by interferon; Blood Mx1 levels in GVHD did not differ from controls ¹¹
interferon-induced GTP-binding protein Mx2	31	29.8	Similar structure to Mx1; known to be upregulated in the viral response; not previously studied in GVHD, to our knowledge
interferon-induced guanylate-binding protein 2	29.7	29.4	Known to inhibit cytotoxicity of paclitaxel by blocking apoptosis; not previously studied in GVHD, to our knowledge
myeloid cell nuclear differentiation antigen	29.8	29.1	Interferon-induced transcription factor expressed on monocytes and granulocytes; not previously studied in GVHD, to our knowledge
Antigen presentation/processing			
antigen peptide transporter 1	31.1	31.3	Transporter of cytosolic peptides to the endoplasmic reticulum; facilitates loading onto HLA; not previously studied in GVHD, to our knowledge
proteasome subunit alpha type-3 isoform 1	32.3	30.4	Early administration of the proteasome inhibitor bortezomib can inhibit GVHD ¹²
signal transducer and activator of transcription 3 isoform 2	31.3	29.4	Downstream protein of IL-22, a cytokine known to worsen end-organ damage in acute GVHD ¹³
Cellular adhesion and signaling			
integrin beta-2 (CD18) precursor	29.7	29.4	In a murine allo-GVHD model, mice that received CD18 ^{-/-} donor T-cells had significantly decreased rates of GVHD morbidity and mortality ¹⁴
LIM domain and actin-binding protein 1 isoform 2	31.3	29	Co-localizes to integrin adhesion sites of keratinocytes; not previously studied in GVHD, to our knowledge
Respondents to cellular stress			
heat shock protein 105 kDa	29.5	29.2	Though little is known about HSP 105, HSP90 inhibitor appears to provide a protective effect against acute gastrointestinal GVHD in murine models ¹⁵
hypoxia up-regulated protein 1 precursor	32.9	29.8	Cellular endoplasmic reticulum chaperone belonging to the heat shock protein 70 family
sulfide:quinone oxidoreductase, mitochondrial	30.9	30.2	Enzyme that functions in the initial step of hydrogen sulfide oxidation and regulates smooth muscle tone, including that of the cutaneous microvasculature; not previously studied in GVHD, to our knowledge

Note: No protein was significantly overexpressed in negative controls over allogeneic graft-versus-host disease (GVHD) or autologous GVHD.

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